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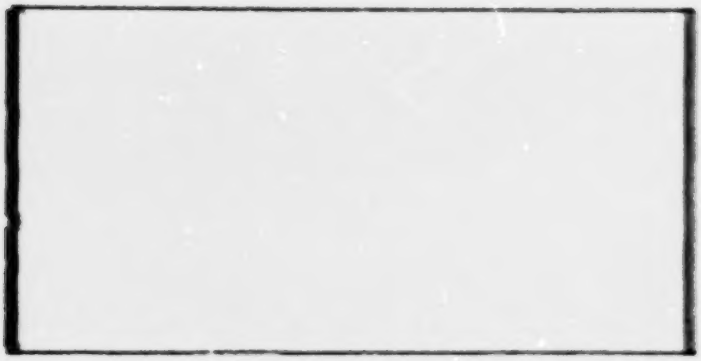

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# TRITIATED WATER UPTAKE KINETICS IN TISSUE-FREE WATER AND ORGANICALLY-BOUND FRACTIONS OF TOMATO PLANTS

Report No 84-69-K

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## ABSTRACT

The kinetics of tritiated water (HTO) vapour uptake into tissue-free water tritium (TFWT) and organically bound tritium (OBT) fractions of tomato, *Lycopersicon esculentum* Mill., cv Vendor, were investigated under controlled growing conditions. Most uptake data fitted a first-order kinetic model, $C_t = C_\infty (1-e^{-kt})$, where $C_t$ is the tritium concentration at time t, Ca the steady-state concentration and k the uptake rate constant. During atmospheric-HTO exposure with clean-water irrigation in open pots the TFWT k values were 0.024 ± 0.023 $h^{-1}$ for new foliage, 0.104 ± 0.067 $h^{-1}$ for old foliage and 0.042 ± - 0.136 $h^{-1}$ for new green fruit. OBT uptake rate constants were 20% less for new foliage and 76% less for new green fruit. Under steady-state conditions the ratio of tritium specific activities of TFWT to atmospheric HTO were 0.43 in new foliage, 0.46 in old foliage and 0.19 in green fruit. Within the plant, OBT to TFWT ratios were 0.70 for new foliage, 0.63 for old foliage (maximum) and between 0.72 and 1.92 for green fruit. The greater than unity tritium specific activity ratios in green fruit were not attributed to tritium enrichment but rather to the translocation of foliar OBT to the growing fruit which contained lower specific activity TFWT derived from soil water.



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EXECUTIVE SUMMARY

# TRITIATED WATER UPTAKE KINETICS IN TISSUE-FREE WATER AND ORGANICALLY-BOUND FRACTIONS OF TOMATO PLANTS

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Chemical Research Department

Tritium, the only radioisotope of hydrogen, is produced in CANDU heavy water reactors by the interaction of neutrons with deuterium in moderator and primary heat transport systems. Present CANDU reactor operations result in the release of tritium in both the vapour and liquid states to the aquatic and terrestrial environments. Although tritium is the most abundant radionuclide released, emissions are well within the limits prescribed by the Atomic Energy Control Board.

Prediction of tritium behaviour in the environment and in human dose-modelling is commonly based on the assumptions of the tritium specific activity model. In this model the specific activity, expressed here as tritium radioactivity per unit mass of hydrogen, in a receptor organism is assumed to be equal to (worst case) or less than the specific activity of the exposure source, as for example in the atmosphere or aquatic media. Since tritium, as a radioisotope of hydrogen, may replace the latter during biosynthesis of organic constituents in living organisms, it is likewise assumed under the specific activity model that the specific activity of the organic constitutents, commonly termed organically-bound tritium, would be equal to or less than the body fluids or tissue-free water tritium. These assumptions imply the absence of tritium enrichment or bioaccumulation either within organisms or between organisms in food-chains. However, concerns have been raised about the general application of the assumptions of the tritium specific activity model because specific activities exceeded unity in certain reports on environmental samples.

In the present experimental study a plant species, tomato, was exposed to atmospheric tritiated water under controlled conditions to determine 1) the rate of tritium uptake in foliage and fruit 2) the tritium specific activity relationships between

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the atmospheric-HTO source and tritium in tissue-free water and organically-bound constituents and 3) specific activity relationships between tissue-free water and organically-bound tritium fractions within the tomato.

The results demonstrated the rapid uptake of tritiated water into tomato tissue-free water, but slow incorporation into organically-bound constituents of growing tissues. The tritium specific activity ratios of tissue-free water tritium to atmospheric source and organically-bound tritium to source support the assumptions of the specific activity model. The observations of less than unity ratios in foliage, but elevated organically-bound to tissue-free water tritium specific activity ratios (0.72 to 1.92) in fruit, were explained not on the basis of tritium enrichment, but on the normal export of foliar-photosynthetic building blocks to the fruit where tissue-free water was comparatively tritium-free. This information serves to explain the elevated specific activity ratios (3 to 5) observed in greenhouse vegetables grown near a CANDU reactor.

## TABLE OF CONTENTS


| | | Page |
|---|---|---|
| 1.0 | INTRODUCTION | 1 |
| 2.0 | METHODS | 3 |
| | 2.1 Plant Stock and Exposure Conditions | 3 |
| | 2.2 Monitoring and Analytic Methods | 5 |
| 3.0 | RESULTS AND DISCUSSION | 7 |
| 4.0 | CONCLUSIONS | 20 |
| 5.0 | REFERENCES | 23 |
| 6.0 | APPENDIX | 27 |
| DISTRIBUTION | | last page |

Page

## LIST OF FIGURES


| | | |
|---|---|---|
| FIGURE 1 | Controlled Environment Chamber Layout | 4 |
| FIGURE 2 | HTO Uptake in Tomato Fruit and Foliage TFWT During Exposure to Tritiated Water Vapour | 9 |
| FIGURE 3 | HTO Uptake in Tomato Fruit and Foliage OBT During Exposure to Tritiated Water Vapour | 10 |
| FIGURE 4 | Changes in Tritium Specific Activity Ratio of OBT to TFWT during HTO Uptake | 18 |


## LIST OF TABLES


| | | |
|---|---|---|
| Table I | HTO Uptake Kinetics in Tomato Fruit and Foliage During Exposure to Tritiated Water Vapour | 11 |
| Table II | Stomatal Diffusion Resistance | 13 |
| Table III | Tritium Specific Activity Relationships in Tomato Exposed to Atmospheric Tritiated Water Vapour | 14 |


## APPENDIX


| | | |
|---|---|---|
| Appendix 1 - | Tritiated Water Uptake Kinetics in TFWT and OBT Fractions of Tomato Foliage and Fruit | 27 |

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To Mr. F.J. Kee
Director of Research

# TRITIATED WATER UPTAKE KINETICS IN TISSUE-FREE WATER AND ORGANICALLY BOUND FRACTIONS OF TOMATO PLANTS

## 1.0 INTRODUCTION

Tritium, the only radioisotope of hydrogen, with atomic mass of three is produced in CANDU pressurized heavy water reactors by the interaction of neutrons with deuterium (heavy water) in the moderator and primary heat transport systems. Present CANDU operations result in the release of tritium in the form of tritiated water, HTO, in both liquid and vapour states. This tritium which constitutes the most abundant radionuclide emission, a characteristic of heavy water reactors, is released well within limits prescribed by the Atomic Energy Control Board. Future tritium emissions will include elemental tritium ($T_2$/HT/DT) once tritium recovery systems are constructed in the mid-eighties to reduce tritium concentrations in heavy water systems. Other anthropogenic sources of tritium include alternatively-designed fission-reactors for power generation, fuel reprocessing plants, commercial tritium applications as in lighting devices and, most important in terms of abundance, the earlier detonation of nuclear explosives in the period from 1945 to 1975 (Till *et al*, 1980; National Council on Radiation Protection and Measurements, 1979).

The results reported here form part of a Biological Research Tritium Program initiated in 1980 by the Chemical Research Department in support of Ontario Hydro's nuclear generation program. The goal of this program is to provide data which will enable modelling the fate and behaviour of tritium in the natural environment. In this particular report controlled environment experiments were undertaken in an attempt to clearly establish the tritium specific activity relationships (1) between atmospheric sources of HTO and the receptor organism, in this case a higher plant, tomato, and (2) between tritium in tissue-free water tritium (TFWT) and organically bound tritium (OBT) under chronic exposure conditions. Such conditions parallel the chronic release situations characteristic of operating CANDU facilities.

Results of environmental sample analysis have raised some questions over the generalized application of the assumptions of the tritium specific activity model whereby specific activity ratios of unity or less are expected in biotic receptors or various trophic levels of ecosystems relative to exposure or food sources of tritium. For example the tritium concentration relationships, expressed or recalculated on a gram hydrogen basis, for organically bound fractions (OBT) versus tissue-free water (TFWT) or exposure medium have been reported as greater than unity for a number of locations. Bogen and Welford (1976) and Bogen *et al* (1979) measured for general environmental sources OBT/TFWT ratics ('bound' to 'loose') of 6 to 14 for soils, 1.8 to 4 for human food including vegetables and 1.5 to 2 for human tissues. Cohen and Kneip (1973) reported values of 2 for pond weed and 5 to 6 for river sediments at a NE United States pressurized-water reactor site and König and Winter (1977) values of 2 to 10 for foliage of pine, hornbeam and spruce at a European nuclear research facility. A similar range of values, 2 to 7, was measured in greenhouse grown tomato and cucumber fruit grown at a CANDU reactor site (Gorman, 1980). At other European nuclear and radiochemical sites Kirchmann (1979) has reported values of 2 to 51 for sediments, 3 to 36 for aquatic plants and 5 to 24 for aquatic animals sampled in liquid effluents. When algae were cultured in these effluents the tritium specific activity ratios were 26 to 54 at the radiochemical site but much less at the PWR sites, 0.9 to 18. The elevation of ratios beyond unity was attributed to potential release of tritiated organic substances (Kirchmann *et al*, 1979).

At two formerly active nuclear test sites, Eniwetok atoll and Nevada, tritium specific activity ratios for OBT relative to TFWT were greater than unity in soils, wood and aquatic animals (Koranda, 1965; Martin and Koranda, 1972). However, in most environmental samples ratios of specific activities of OBT to TFWT or source tritium have been near or less than unity as in aquatic organisms reviewed by Bruner (1973) and in woody plant species examined by Brown (1979). Potential explanations for elevated organically-bound tritium values relative to tissue-free water or source tritium in environmental samples include temporal variation in source concentrations particularly decline in exposure levels, differential turnover rates of TFWT and OBT fractions within organisms and isotopic discrimination which may occur during biosynthetic and degradative stages of metabolism. These ideas have been supported by controlled environment experiments. For example, rapid turnover of TFWT relative to OBT following short-term (0.5 to 1 h) atmospheric HTO exposure in field-grown corn, sunflower, burclover and black pine lead to OBT/TFWT ratios which increased with time in all species reaching a maximum of about 100 in corn and sunflower (Koranda and Martin, 1973). Evidence for isotopic discrimination of tritium in plant species has been reviewed by McFarlane (1976).

The present study demonstrates that even with controlled atmospheric-HTO exposure, tritium specific activity ratios (OBT/TFWT) may exceed unity in certain organs of higher plants.

## 2.0 METHODS

### 2.1 Plant Stock and Exposure Conditions

Tomato seeds, *Lycopersicon esculentum* Mill., cultivar Vendor, were surface sterilized with 0.5% NaOCl, rinsed in tap water then germinated in flats containing Redi-Earth, a commercial mixture of equal quantities of peat and vermiculite containing starter fertilizer.

When 14 to 16-weeks old, 6 plants were used for atmospheric HTO exposure experiments in a Conviron Model E15 plant growth chamber. The plants were placed in the exposure chamber after a steady-state atmospheric-HTO concentration had been reached (95 080 ± 11 280 pCi/g H, $\bar{x}$ ± sd, n = 39).

Prior to HTO exposure, plants were acclimated at least 24 h to the exposure regime of 16 h light at 23° ± 1°C, 70 ± 2% RH and 8 h dark at 18° ± 1°C, 62 ± 2% RH. Light intensity measured as photosynthetic photon flux density was 500 $\mu E/s/m^2$ at the beginning of the experiment and 430 $\mu E/s/m^2$ at the end. These measurements were made at the top of the plant canopy using a Lambda instrument, Model L1-185A, with quantum sensor, L1-190S.

Delivery of HTO vapour to the growth chamber atmosphere was accomplished by passing a dry air stream at 75 cc/min through a gas washing bottle containing 125 µCi/mL, maintained at 15°C in a water bath. Tritiated water in 5 mCi/mL aliquots was purchased from Amersham. The exposure apparatus is outlined in Figure 1, with a complete description provided in Ontario Hydro Research Division Report No 83-33-K (1983a).

Irrigation consisting of 2 L half-strength Hoagland's solution (Langhans, 1978) was supplied twice daily to each pot. This provided a slight excess which curtailed salt accumulation in the soil and allowed collection of soil water for HTO measurements. Carbon dioxide concentrations in the growth chamber during the light period were maintained at 400 ± 25 $mm^3/m^3$ by delivery of 35 to 40 cc/min commercial grade (99.5%) $CO_2$. During the dark cycle $CO_2$ concentrations increased to a maximum of about 650 $mm^3/m^3$ due to plant respiration. Carbon dioxide was measured continuously with a Beckman infrared analyzer, Model 864.

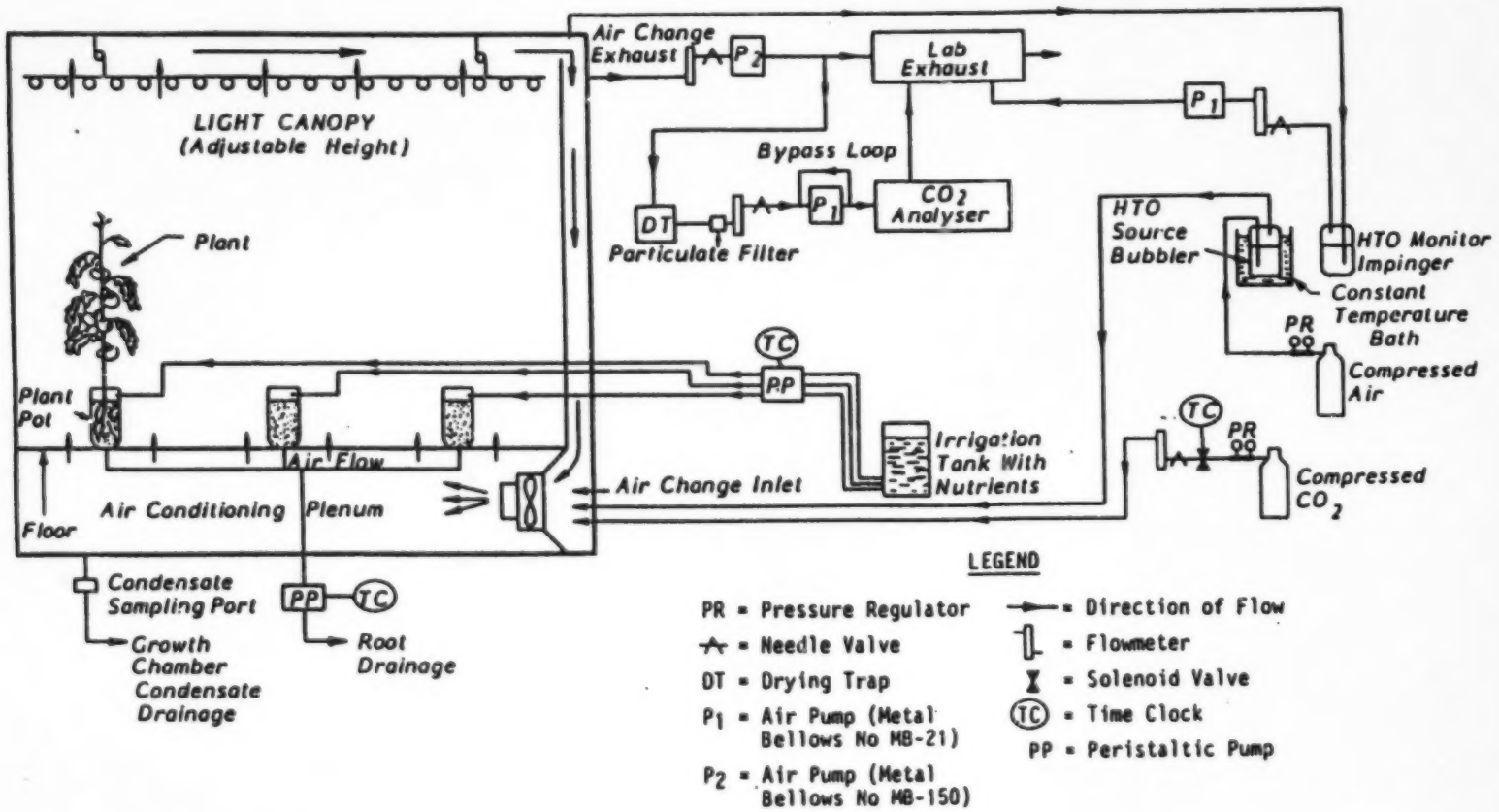


FIGURE 1

CONTROLLED ENVIRONMENT CHAMBER LAYOUT
(DIAGRAM SHOWS ONLY 3 OF 6 PLANTS AND IRRIGATION LINES UTILIZED)

## 2.2 Monitoring and Analytic Methods

### 2.2.1 Measurement of Chamber Atmospheric HTO Concentration

The concentration of tritium in water vapour of the growth chamber atmosphere was measured in condensate collected from refrigeration coils responsible for chamber cooling and dehumidification. This condensate, resulting from plant transpiration and chamber dehumidification processes, was formed at a rate of about 1L/h. Tritium activity in the condensate was measured by liquid scintillation counting using PCS II cocktail (Amersham) with a sample/cocktail ratio of 1:14.

A correction factor, 0.90, was applied to atmospheric condensate data because earlier measurements had shown the tritium concentrations in condensate to be overestimated by about 10.3% compared to values obtained by direct collection of chamber air in liquid scintillation cocktail (PCSII) (see Appendix IV, Ontario Hydro Research Division Report No 83-33-K, 1983a). Similar differences in tritium concentrations between growth chamber condensate and either freeze-trapped or molecular seive-trapped atmospheric HTO were reported by McFarlane *et al* (1979).

### 2.2.2 Stomatal Diffusion Resistance

Stomatal diffusion resistance, $r_s$, was measured to ensure that the potential for water vapour transfer and hence HTO movement was normal and evenly controlled over the HTO exposure period, given the specific exposure conditions (Section 2.1). Regulation of stomatal diffusion resistance is a most important variable in controlling tritium uptake rates.

Stomatal diffusion resistance during the light portion of the photoperiod was measured on the upper surface of randomly selected old and new foliage from six plants using an autoporometer, Lambda Instruments Model L165. For each sampling period the mean values were calculated, n = 6 (Table III) after standardization at 25°C. The autoporometer was calibrated with moistened filter paper placed on a calibration plate having five sets of pores of known size and number (Lambda Instruments No 201S).

### 2.2.3 Foliage and Fruit Sampling

For sampling purposes new foliage was that formed during HTO exposure and old foliage was that formed before exposure but which underwent some leaf expansion and cell wall thickening during exposure. Fruit samples consisted primarily of new growth (>90% fresh wt.) because all fruit greater than 5 mm diameter were removed before HTO exposure.

On each sampling occasion, except zero time, old and new foliage was sampled randomly from 3 of 6 plants, then composited before extraction of tissue-free water tritium (TFWT) and organically-bound tritium (OBT). Unlike foliage, fruit was sampled in triplicate by selecting fruit from 3 of 6 plants on each sampling occasion. Previous experiments involving TFWT measurements had shown low variability in foliage sampling, typically 1 to 5% coefficient of variation, while green fruit exhibited high variability, typically 20 to 40% (Ontario Hydro Research Division Report No 83-33-K, 1983a).

### 2.2.4 Tissue-Free Water Tritium Analyses

Tissue-free water tritium in fruit and foliage was extracted quantitatively with an azeotropic toluene-water mixture (bp = 85°C) to provide 5 to 10 mL of tissue-free water, a modification of the procedure described by Moghissi *et al* (1973). This procedure is utilized by Ontario Hydro Health and Safety Division for environmental samples. Approximately 10 to 30 g fresh weight of tomato material were extracted with 2 to 3 volumes of liquid scintillation grade toluene. The distillate was analyzed routinely for tritium by adding 1 mL of sample to 14 mL of liquid scintillation cocktail, PCS II, then counting in a Searle MKIII liquid scintillation counter. During TFWT analyses a daily control, consisting of 30 mL well water containing less than 170 pCi/mL tritium activity, was run through the distillation apparatus.

### 2.2.5 Organically Bound Tritium Analysis

Sample residues remaining after azeotropic toluene distillation of TFWT were air dried to remove most toluene, then dried to constant weight *in vacuo* over phosphorus pentoxide. For each dried sample 2 to 3 g were pelletized (about 1 g/pellet) and redried to constant weight. Pellets were combusted using a Packard Tri-Carb Oxidizer, Model C306, to recover organically bound tritium. Combustion water was collected in 15 mL of Monophase 40 liquid scintillation cocktail. Tritium radioactivity was measured in a Packard Tri-Carb 300 liquid scintillation counter. Care was taken to avoid contamination of low activity samples by carryover from high activity material. During the processing of high activity samples a blank consisting of a combustion cup alone was oxidized to check for carryover. These blanks were typically 15 to 25 pCi before background correction (about 14 pCi).

### 2.2.6 Hydrogen Determination

To provide an equivalent basis for comparing tritium activity in tomato TFWT and OBT fractions, activity was expressed per gram hydrogen of sample.

Hydrogen content of organic samples was determined by a gravimetric procedure on non-tritiated foliage and green fruit grown under conditions described for HTO exposure. Old and new foliage and green fruit were sampled from three plants. From 2 to 5 g of sample dried to constant weight were used for analysis (Analytic Services Section).

### 2.2.7 Data Analysis

Tritium uptake data was fitted by a computerized non-linear least squares method (NLIN procedure of Statistical Analyst Systems, SAS Institute, Cary, NC) to provide steady-state concentrations, $C_\infty$, and uptake rate constants, k, from which initial uptake rates, $C_{\infty k}$, and the times to reach 50% of the steady-state concentration, $t_{1/2}$, could be calculated. Most uptake data was found to follow first-order kinetics, whereby it was assumed uptake would be proportional to exposure concentration. The following equation was used to represent these uptake patterns:

$$C_t = C_\infty (1-e^{-kt})$$

where

$C_t$ = tritium concentration at time t, expressed in pCi/g hydrogen of sample
$C_\infty$ = steady-state concentration (asymptotic value) reached when dC/dt = 0
t = time in hours
k = initial uptake rate constant at t=0, expressed in $h^{-1}$
and
$t_{1/2}$ = 0.693/k, the time required to reach one-half of the steady-state concentration, expressed in hours.

The computer estimates of the steady-state concentrations and initial uptake rate constants are for each parameter accompanied by a 95% confidence interval ($\sigma t$). Manual calculations were used to estimate the chamber atmospheric HTO steady-state concentration and maximum value reached for OBT in old foliage.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Tissue-Free Water Tritium Kinetics (TFWT)

In the experiments discussed below whole tomato plants, grown in open containers, were exposed to tritiated water vapour in a controlled environment chamber in which light intensity, temper-

ature and relative humidity were held constant. Regular irrigation schedules were followed and carbon dioxide levels controlled during the daylight photoperiod, but allowed to rise in the dark period due to respiration. These conditions, as described in Methods, were intended to reflect field situations where soil tritium levels are near background and atmospheric tritium concentrations are elevated.

Uptake kinetics of tri iated water vapour into tissue-free water tritium (TFWT) fractions of tomato foliage and fruit are shown in Figure 2. The corresponding data for HTO uptake into the organically-bound tritium (OBT) fraction are displayed in Figure 3. In this experiment the chamber atmosphere was brought to a steady-state tritium concentration before introducing plants into the chamber. During exposure the steady-state tritium concentration (average) was 10 900 ± 1260 pCi/mL of atmospheric moisture ($\bar{x}$ ± sd, n = 39). This concentration was equivalent to 97 580 ± 11 280 pCi/g hydrogen which was the basis used for comparing TFWT and atmospheric HTO to OBT data throughout this report.

The factor for converting TFWT data expressed in pCi/mL (Appendix I) to pCi/g hydrogen was 8.954 based on atomic mass ratios and density of water at 20°C. Organically-bound tritium (OBT) data measured in pCi/g dry weight (Appendix I) was converted to an equivalent gram hydrogen basis using the following measured hydrogen content values: upper foliage 60 ± 5 g/kg, lower foliage 54 ± 2 g/kg, and green fruit 66 ± 7 g/kg ($\bar{x}$ ± sd, n = 4, g hydrogen/kg dry weight).

During sampling for tritium measurements the foliage was separated into two categories, old and new. Although new foliage was formed only on the upper portions of each plant after exposures started, old foliage present on the lower portions of the plants likely involved some new growth through leaf expansion and cell wall thickening.

Tritiated water vapour uptake appeared to be most rapid in old foliage, followed by new foliage and green fruit (Figure 2) similar to earlier observations for foliage (old and new, not separated) and fruit (Ontario Hydro Research Division Report No 83-33-K, 1983a). However, after fitting data by a computerized non-linear least squares method to a pattern following first-order kinetics the uptake rate constants could not be declared significantly different since they overlapped using 95% confidence intervals. The uptake rate constants were 0.024 ± 0.023 $h^{-1}$ for new foliage, 0.104 ± 0.067 $h^{-1}$ for old foliage and 0.042 ± 0.136 $h^{-1}$ for green fruit (Table I). Previously, with similar exposure conditions, larger rate constants were observed for foliage, 0.700 ± 0.148 $h^{-1}$ and 0.708 ± 0.288 $h^{-1}$, but a

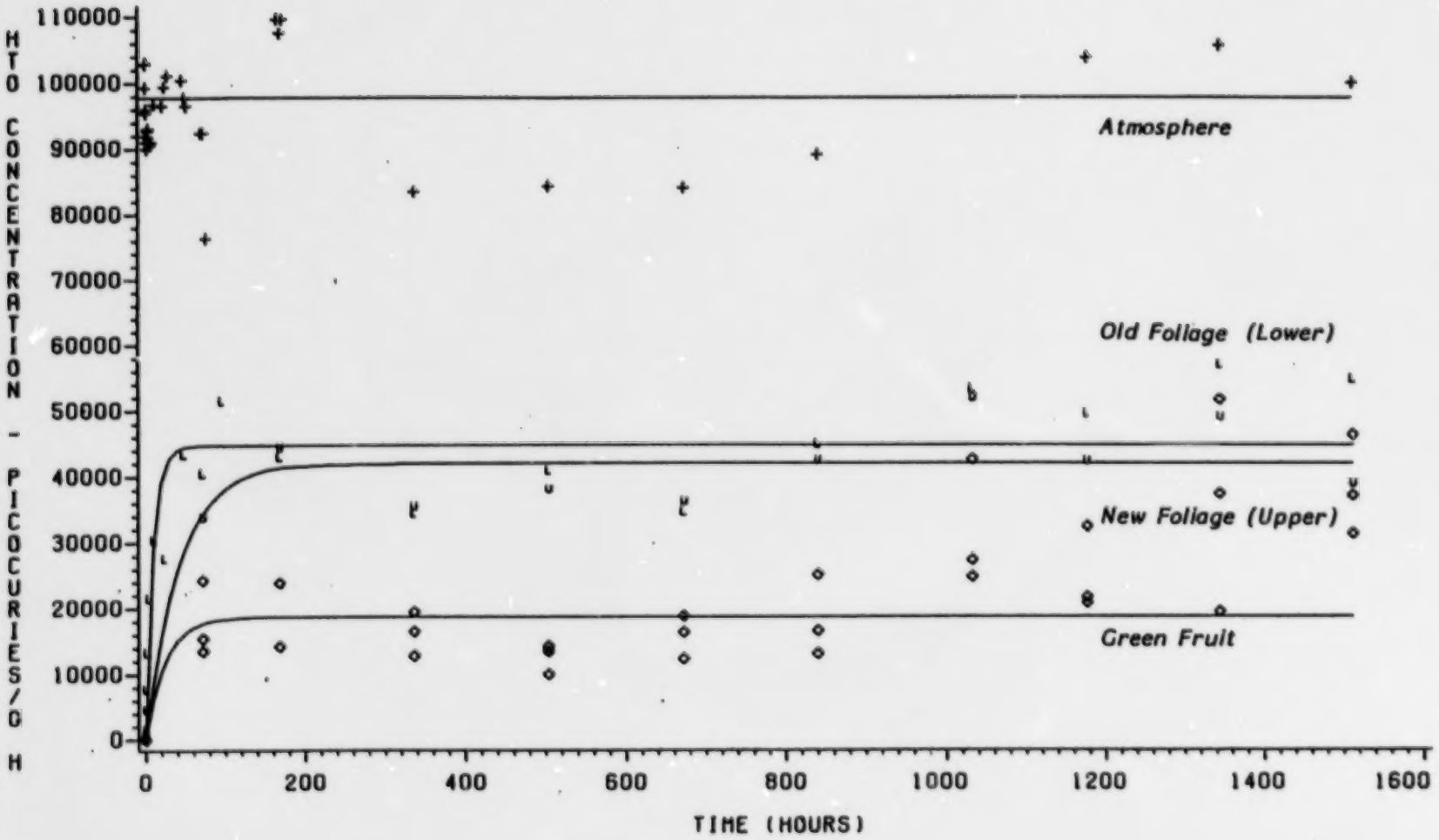


FIGURE 2
HTO UPTAKE IN TOMATO FRUIT AND FOLIAGE TFWT
DURING EXPOSURE TO TRITIATED WATER VAPOUR

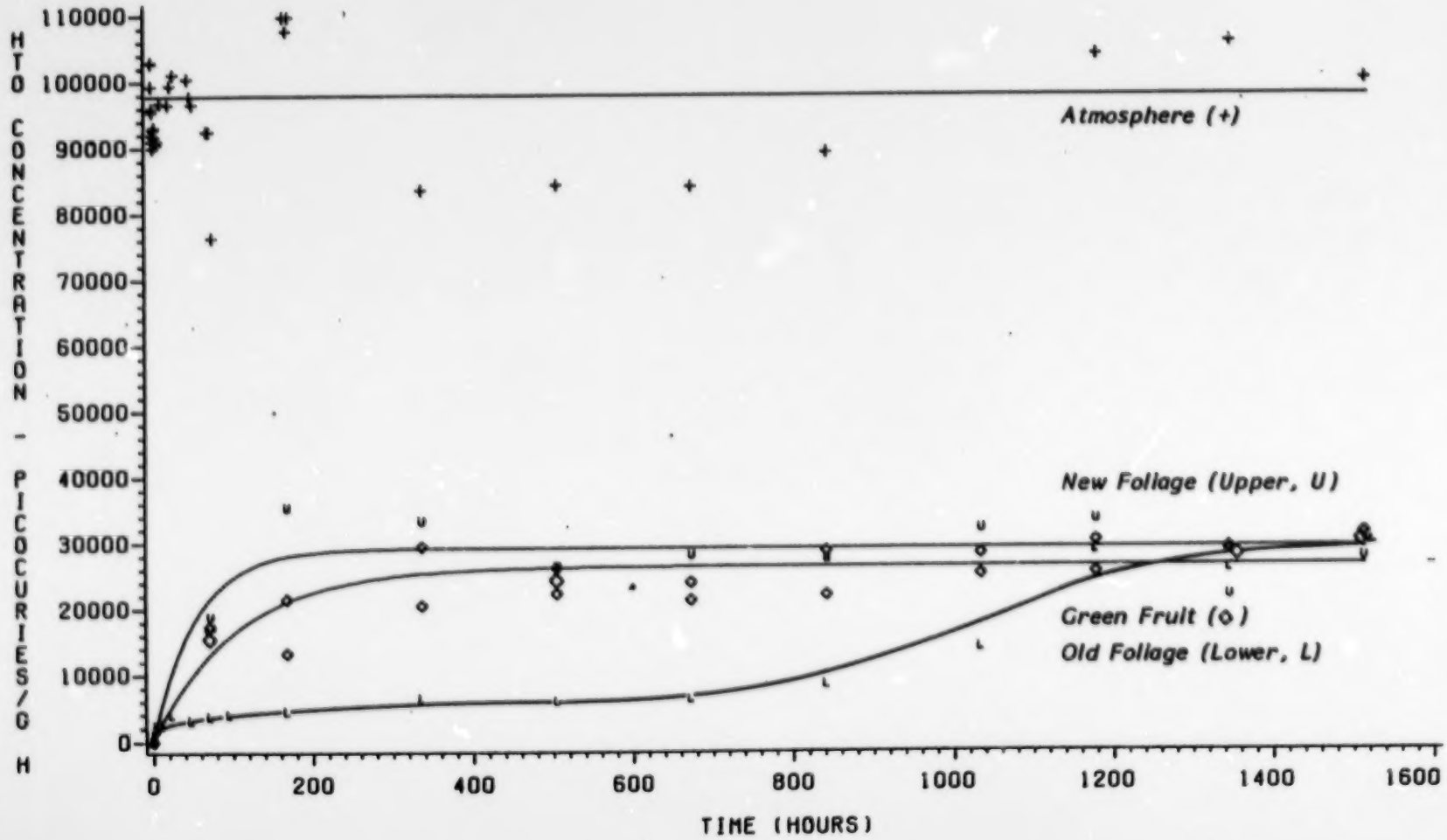


FIGURE 3
HTO UPTAKE IN TOMATO FRUIT AND FOLIAGE OBT
DURING EXPOSURE TO TRITIATED WATER VAPOUR

TABLE I

HTO UPTAKE KINETICS IN TOMATO FRUIT AND FOLIAGE
DURING EXPOSURE TO TRITIATED WATER VAPOUR

| | Tritium Fraction | Steady-State Conc'n, $C_\infty$ (pCi/g H) | Uptake Rate Constant, k ($h^{-1}$) | Initial Uptake Rate, $C_\infty k$ (pCi/g H/h) | 50% Steady-State Conc'n, $t_{1/2}$ (h) |
|---|---|---|---|---|---|
| New Foliage | TFWT | 41 970 ± 4160 | 0.024 ± 0.023 | 1000 | 29 |
| | OBT | 29 150 ± 3460 | 0.019 ± 0.018 | 550 | 37 |
| Old Foliage | TFWT | 44 800 ± 4240 | 0.104 ± 0.067 | 4660 | 7 |
| | OBT | unattained | - | - | - |
| Green Fruit | TFWT | 18 660 ± 3480 | 0.042 ± 0.136 | 780 | 17 |
| | OBT | 26 330 ± 2000 | 0.010 ± 0.004 | 260 | 69 |
| Soil Water | TFWT | 2990 ± 750 | 0.057 ± 0.300 | 170 | 12 |

Steady-state tritium concentrations and initial uptake rate constants (estimate ± 95% confidence interval) were estimated by fitting data to the equation $C = C_\infty(1-e^{-kt})$. Tritium uptake rate curves are shown in Figures 2 and 3.

similar value for green fruit, 0.060 ± 0.037 $h^{-1}$ (Ontario Hydro Research Division Report No 83-33-K, 1983). In bean foliage values of 0.58 ± 0.38 ($\bar{x}$ ± 95% confidence limits, n = 15) were reported by Garland and Cox (1982). The high variability, particularly of fruit TFWT data, had not allowed separation of the fruit and foliage rate constants as expected. There was an upward drift in fruit TFWT concentrations between 1032 and 1512 h which was proportionately much greater than the increase in either foliar TFWT or atmospheric-HTO concentrations (Figure 2). Likewise the small changes in soil water tritium did not account for the rising trend in fruit TFWT (Appendix I). Normally, high variability, typically 20 to 40% coefficient of variation, has been observed in tomato fruit TFWT, unlike the low variability typically 5% associated with foliage (Table III, Ontario Hydro Research Division Report No 80-52-K, 1980).

The potential for tritiated water vapour uptake was maintained at a constant level during the course of experiments reported here. This was accomplished by establishing a constant vapour pressure deficit, 0.85 kPa, between the atmosphere and the foliage interior during both the day and night portions of the thermal-period (23°C day/18°C night). It has been assumed in these calculations that the leaf interior (stomatal cavities and mesophyll cells) was at 100% relative humidity. Given a constant driving force for HTO vapour, measurement of stomatal diffusion resistance to water vapour has provided indications that factors which regulate stomatal gas transfer (light intensity, temperature, vapour pressure deficit, carbon dioxide levels, stage of development; see Jarvis and Mansfield, 1981; Schulze and Hall, 1982) have remained reasonably constant (Table II). Stomatal diffusion resistance measured only on upper leaf surfaces exhibited no particular trends in either old or new leaves. Similar relationships would be expected for the lower surfaces of old and new foliage. The relative resistance of older foliage to water vapour transfer is reflected in their high values ranging from 4.3 to 13.6 s/cm compared to new leaf numbers of 1.7 to 3.1 s/cm which are normally associated with high transpirational and photosynthetic activities (Table II).

Clear patterns did emerge in the differences between steady-state TFWT concentrations of each of the plant organs and the chamber atmospheric HTO. With steady-state concentrations of 41 970 ± 4160 pCi/g hydrogen in new foliage, 44 800 ± 4240 pCi/g hydrogen in old foliage and 18 660 ± 3480 pCi/g hydrogen in green fruit, the ratios of the tritium specific activities of new foliage, old foliage and fruit relative to the chamber atmosphere became 0.43, 0.46 and 0.19, respectively (Table III). These ratios were in good agreement with earlier experimental findings in this laboratory: 0.45 for foliage/

## TABLE II

## STOMATAL DIFFUSION RESISTANCE

The stomatal diffusion resistance, $r_s$, of the top leaf surfaces of randomly selected foliage of six tomato plants was measured. Values are shown as mean ± std dev, n = 6, corrected to 25°C.

| HTO Exposure Duration (h) | Stomatal Resistance, $r_s$ (s/cm) | |
|---|---|---|
| | New Leaves | Old Leaves |
| 0 | 2.6 ± 0.5 | 13.6 ± 1.3 |
| 72 | 2.4 ± 1.0 | 12.5 ± 5.7 |
| 168 | 2.6 ± 0.8 | 9.1 ± 6.7 |
| 336 | 3.0 ± 1.1 | 9.5 ± 7.1 |
| 504 | 3.1 ± 1.7 | 4.7 ± 2.0 |
| 672 | 1.7 ± 1.1 | 4.9 ± 2.3 |
| 864 | 2.1 ± 0.9 | 4.3 ± 2.3 |
| 1176 | 2.1 ± 0.9 | 4.3 ± 2.9 |
| 1344 | 2.7 ± 1.6 | 4.4 ± 2.2 |
| 1536 | 3.0 ± 1.8 | 5.6 ± 2.4 |

## TABLE III

### TRITIUM SPECIFIC ACTIVITY RELATIONSHIPS IN TOMATO EXPOSED TO ATMOSPHERIC TRITIATED WATER VAPOUR

Specific activity relationships between the atmospheric HTO exposure source and tomato fruit and foliage tritium, and between the OBT and TFWT fractions within tomato were calculated from steady-state concentration estimates in Table I. The average atmospheric HTO concentration during exposure was 97 580 ± 11 280 pCi/g hydrogen ($\bar{x}$ ± 1 sd, n=39).

| | Atmospheric Source/ Tomato Tritium Fraction | | Within Tomato |
|---|---|---|---|
| | TFWT/Atmosphere | OBT/Atmosphere | OBT/TFWT |
| New Foliage | 0.43 | 0.30 | 0.70 |
| Old Foliage | 0.46 | 0.29* | 0.63* |
| Green Fruit | 0.19 | 0.27 | 1.41 |

* Because a steady-state OBT concentration for lower leaves was not clearly attained, an approximate value of 28 140 ± 2390 pCi/g hydrogen (x ± sd for 1176, 1344 and 1512 h, Appendix I) was used in these specific activity ratio estimates.

atmosphere, 0.31 for green fruit/atmosphere and 0.19 for ripe fruit/atmosphere and in other laboratories (Koranda and Martin, 1973; Kline and Stewart, 1974; and IAEA, 1981). For a similar relative humidity, 70%, a higher ratio of 0.77 was calculated from foliar TFWT steady-state concentrations predicted by recent models of Belot et al (1979) and Garland and Cox (1982). Their models predict that TFWT of foliage would equilibrate with atmospheric HTO concentrations, taking into account the isotopic discrimination factor ($\alpha$ = 1.1) for HTO in liquid and vapour states and relative humidity in air.

In their model terms of the equation $C_t = C_\infty (1-e^{-kt})$ used in fitting present data were expanded to include $C_\infty = \chi\alpha/\rho_{sat}$ and $k = \rho_{sat}/\alpha\mu r$. $\chi$ is the tritium concentration in air expressed in pCi/mL of air, $\alpha$ the isotopic discrimination factor for HTO in liquid versus vapour states, $\rho_{sat}$ the density of water vapour in saturated air in g/mL, $\rho$ the actual water vapour density in air, $\mu$ the amount of water per unit leaf area in g/cm$^2$ and r the foliar resistance to the HTO vapour exchange in s/cm (similar to stomatal resistance, $r_s$, as the boundary layer resistance approaches zero). This model, supported by experimental evidence from foliage of two species, grape and bean, predicts a foliar/atmospheric HTO specific activity ratio of 0.77 at 70% relative humidity.

However, as evidenced in the present and previous studies, equilibration was not reached under constant long-term exposure conditions. Stomatal diffusion resistances of 1.7 to 3.1 s/cm for new foliage and moderate levels of 4.3 to 13.6 s/cm in old foliage were similar to those reported by Belot et al (1979) and Garland et al (1982), suggesting that transpiration rates were not a factor in establishing equilibrium. Such factors are expected to affect the rate constants but not the steady-state TFWT concentrations. Possible explanations for the variation between the Garland and Belot model predictions and the present observations include differences between plant species, an influence of long-term exposures with diurnal cycling as opposed to short-term exposures (1 to 6 h in daylight), and potential effects of leaf water compartmentalization (eg, vascular tissues may contain relatively tritium-free transpiration water; Vaadia and Waisel, 1963; Raney and Vaadia, (1965)).

### 3.2 Organically-Bound Tritium (OBT) Kinetics

During the atmospheric exposure of tomato plants to HTO vapour the concentration of tritium in the OBT fractions of new foliage and green fruit increased rapidly (Figure 3). This was expected given that the new growth would involve labelling primarily through tritium incorporation by biosynthetic reactions into photosynthetic products (sugars, etc) which were then trans-

located as building blocks to the various actively growing regions of the plant. Evidence consistent with this expectation is the fact that similarity was observed between the rate constants for OBT formation in new foliage and fruit and between their steady-state concentrations. In new foliage and fruit the respective rate constants were 0.019 ± 0.018 $h^{-1}$ and 0.010 ± 0.004 $h^{-1}$ while the steady-state concentrations were 29 150 ± 3460 pCi/g hydrogen and 26 330 ± 2000 pCi/g hydrogen (Table I).

Organic constituents in old foliage, unlike new foliage and fruit, were slowly labelled with tritium reaching a maximum OBT concentration of 28 140 ± 2390 pCi/g hydrogen ($\bar{x}$ ± sd for 1176, 1344 and 1512 h sampling points, Figure 3). A steady-state OBT concentration could not be estimated from the available data because of levelling off between 11 and 672 h of exposure followed by a gradual increase to an upper plateau between 1176 and 1512 h. Two mechanisms are proposed for the labelling of organic substances in old leaves. These are tritium-hydrogen exchange between TFWT and labile hydrogens of functional groups of OBT, including -OH, -COOH, $-NH_2$ and -SH, and incorporation of tritium by biosynthetic reactions primarily in photosynthetic products, most of which would be normally translocated to actively growing areas of the plant. The rise in old-foliage OBT concentration with time was likely due principally to an increase in the amount of tritiated photosynthate used locally in leaf expansion and cell wall thickening. It should be noted that the designation old foliage was based on the presence of leaves before HTO exposure and did not preclude either leaf or cell wall thickening, processes that could not easily be delineated in these experiments.

A possible explanation for the old-foliage OBT concentration rising to a second plateau (Figure 3) may be attributed to the sampling of leaves which had originated from the uppermost portion of the stem at time zero and thereby had undergone considerable expansion. The relative contribution of tritium-hydrogen exchange and biosynthetic incorporation processes to tritium labelling in old leaves cannot be distinguished in these experiments. From six-hour double-labelling ($^{14}CO_2$ and HTO) experiments with maize and potato foliage, Belot *et al* (1983) have suggested that tritium labelling occurs by biosynthetic incorporation alone and not by tritium-exchange reactions. This evidence is based on the observations that the ratio, $R_T$, of OBT formed/TFWT correlated linearly ($r = 0.97$) with the ratio, $R_C$, of $^{14}C$-fixed/$^{14}CO_2$ supplied in air and that the ratio $R_T/R_C$ was less than unity (0.37). However, these experiments did not rule out a small degree of tritium-hydrogen exchange, assuming a T-H exchange rate parallelling the photosynthetic rate. The number of exchangeable hydrogens in

organic substances is expected from theoretical calculations to range from 20 to 30%. It is doubtful that most OBT is completely accessible for exchange, particularly structural carbohydrates, lipids and proteins (Lang and Mason, 1960; Leach and Springell, 1962).

The tritium-specific activity ratios of OBT to the atmospheric HTO source under steady-state conditions were 0.30 for upper foliage, 0.29 for lower foliage and 0.27 for green fruit (Table III). These specific activity ratios were in each case about 30% less than the corresponding TFWT to atmospheric HTO ratios. The relationships of OBT to TFWT are discussed in the next section.

### 3.3 Tritium-Specific Activity Ratios in Tomato

The specific activity relationships between OBT and TFWT fractions are compared in Table III and Figure 4. The comparison is based on a per gram hydrogen basis. The ratio of OBT to TFWT increased gradually from zero to about 0.60 in old foliage and from zero to a plateau of 0.70 between 11 and 1512 h exposure in new foliage. These tritium specific activity ratios are generally in the upper range of values reported by other investigators who have continuously exposed higher and lower green plants to HTO in controlled environments. Unless specific substances are listed, the specific activity ratios are for total OBT/TFWT. In higher plants, tritium-specific activity ratios of 0.63 have been reported for maize, 0.54 for barley (Garland and Ameen, 1979), 0.78 for alfalfa (McFarlane, 1976), and 0.39 for peas (Kahna *et al*, 1976). In lower plant species, algae, the following values have been calculated, assuming that TFWT had equilibrated with medium HTO: 0.47 to 0.70 for *Chlorella pyrenoidosa* (Kanazawa *et al*, 1972; Ontario Hydro Research Division Report No 83-383-K, 1983b; Weinberger and Porter, 1953), 0.75 for *Scenedesmus quadricauda* (Strack, 1978) and 0.60 for *Anacystis nidulans* (Rambeck and Bassham, 1973).

In uncontrolled field exposures involving short-term application of HTO by injection, spraying or irrigation, Krishnamoorthy (1979) reported specific activity ratios for OBT to TFWT ranging from 0.001 in terrestrial species to 0.62 in aquatic species. In contrast, in controlled field exposures of corn, sunflower, burclover and black pine to HTO in air, specific activity ratios of up to two orders of magnitude above unity were calculated from data of Koranda and Martin (1973). These high specific activity ratios were attritubed to the rapid turnover of TFWT relative to OBT.

The ratios of OBT to TFWT in green tomato fruit displayed a very irregular pattern during the exposure period (Figure 4). This irregularity was attributed to high variation in the TFWT levels

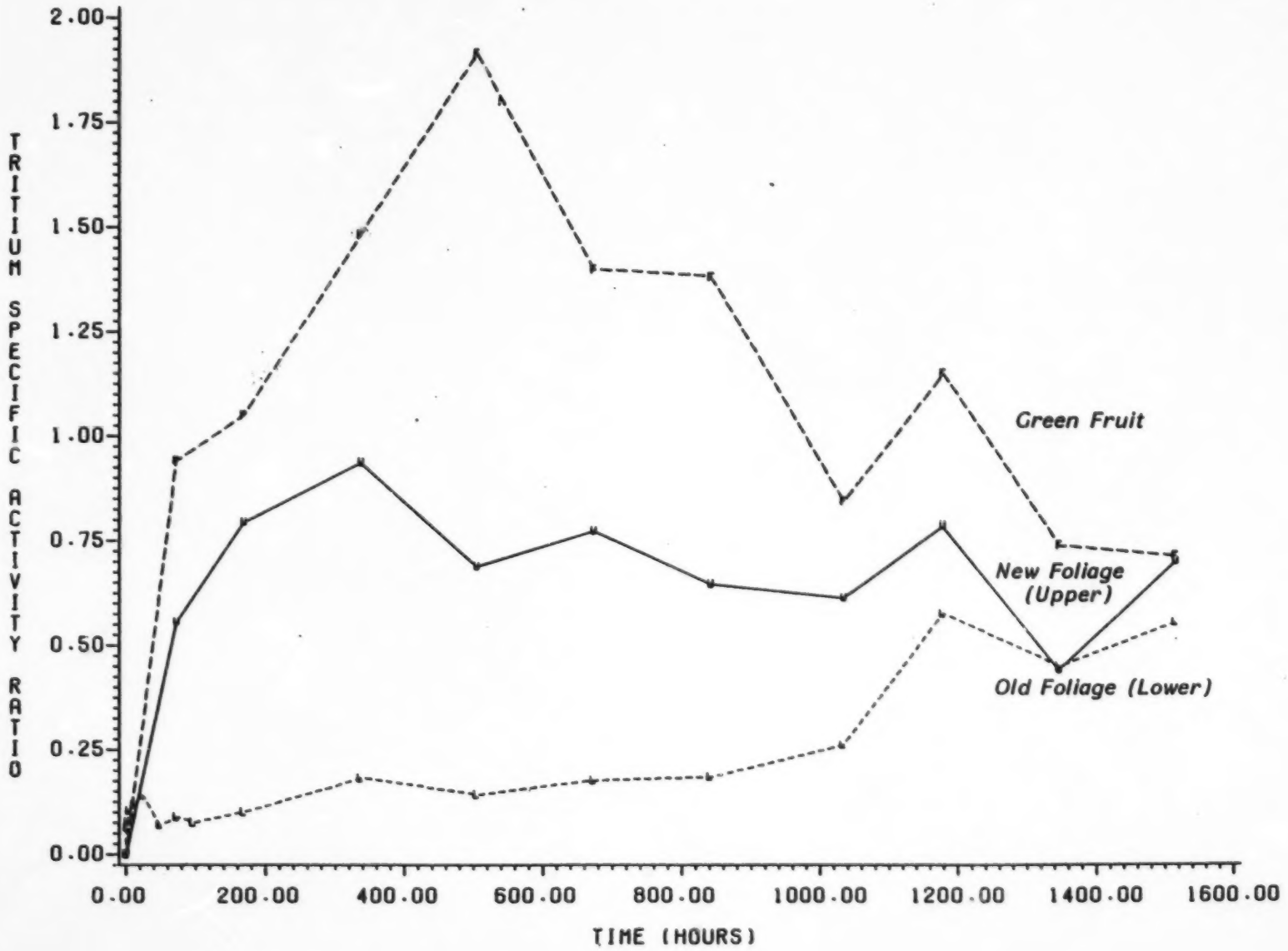


FIGURE4
CHANGES IN TRITIUM SPECIFIC ACTIVITY RATIO
OF OBT TO TFWT DURING HTO UPTAKE

of the green fruit reported earlier (coefficient of variation 20 to 40%, Table III, Ontario Hydro Research Division Report No 80-52-K, 1980). The average specific activity ratio calculated from the computer estimated steady-state TFWT and OBT concentrations (Figures 2 and 3) was 1.41 (Table III) with values ranging from 0.72 to 1.92. If the average ratio is calculated directly from paired OBT/TFWT ratios for each exposure time between 72 and 1512 h, then 1.16 ± 0.38 is obtained ($\bar{x}$ ± sd, n = 10). This is the first occasion in extended controlled tritium exposures where total OBT/TFWT ratios have exceeded unity. These observations are explained not on the basis of a tritium enrichment mechanism, but rather on the extent of foliar and green fruit free-water contamination by atmospheric HTO. The evidence consistent with this interpretation is that TFWT concentrations at steady-state were considerably lower in green fruit than foliage, while OBT steady-state concentrations remained similar in both new green fruit and foliage. The latter relationship would be expected based on the established product-precursor relationship of foliage photosynthate as the primary building-block supply for fruit growth. It is not clear whether isotopic discrimination against tritium was a factor in lower fruit TFWT or whether simply larger proportions of comparatively clean soil water were taken up by fruit than foliage. The steady-state TFWT concentration of tritium in soil water was 2990 ± 750 pCi/g hydrogen, about 16% of that in fruit and 7% of that in new foliage.

Tritium-specific activity ratios exceeding unity have been reported before in metabolic intermediates, but not for total OBT fractions. In *Anacystis nidulans*, a blue-green alga, grown continuously in a tritiated water medium, the citrate/medium HTO ratio was 1.8. In other citric acid or tricarboxylic acid cycle intermediates where organic substances are oxidized and in amino acids derived directly from this cycle ratios were 1.0 or less in both *Anacystis nidulans* and *Chlorella pyrenoidosa*, a green alga (Rambeck and Bassham, 1973; Kanazawa *et al*, 1972). In these species the respective total OBT/medium HTO ratios were 0.60 and 0.70.

The attainment under steady-state conditions of lower tritium-specific activity in OBT than TFWT in new foliage was attributed to isotopic discrimination, similar to the conclusions reached by the above authors (Garland and Ameen, 1979; McFarlane, 1976; Kanazawa *et al*, 1972; Rambeck and Bassham, 1973; Strack, 1978; Ontario Hydro Research Division Report 83-33-K, 1983a and 83-383-K, 1983b). Although the tritium-specific activity of OBT to TFWT in old foliage reached a maximum value of 0.63, the same arguments cannot be made for isotopic discrimination. Non-exchangeable hydrogen as in carbon-hydrogen bonding formed prior to HTO exposure would account for about 70 to 80% of the total hydrogen present at that time.

The data on tomato OBT- to TFWT-specific activity relationships of fruit and foliage exposed to atmospheric HTO offers an explanation for the elevated ratios of tritium-specific activities reported for ripe tomato and cucumber fruit grown in the open environment of a waste-heat prototype greenhouse at a CANDU nuclear generating station. The range of specific activity ratios were 0.72 to 1.92 in the laboratory experiments and 2 to 5 in the greenhouse study (Gorman, 1980). In each case the tritium concentration in soil water was less than the average atmospheric concentration by 2.5 times at the greenhouse location and 33 times in the laboratory study. (The soil water tritium levels averaged 29 pCi/mL at the greenhouse site and 97 500 pCi/g hydrogen in the laboratory study.) Data of OBT to TFWT in new fruit and foliage demonstrated that the greater than unity ratio obtained in fruit was not the result of tritium enrichment in the organic fraction but rather to lower tritium activity in the TFWT fraction, probably a result of comparatively clean soil water uptake.

## 4.0 CONCLUSIONS

Tomato plants were exposed to atmospheric HTO during clean-water irrigation to obtain a better understanding of the tritium uptake kinetics and specific activity relationships between the atmospheric source and tritium in organically-bound (OBT) and tissue-free water (TFWT) fractions of fruit and foliage and of tritium-specific activity relationships between OBT and TFWT fractions. Fourteen- to sixteen-week old plants were exposed to atmospheric tritiated water vapour for a further nine weeks under controlled conditions of temperature, light intensity, carbon dioxide levels, vapour pressure deficit between leaf and atmosphere, and irrigation. The conclusions drawn from these experiments are as follows.

1. HTO uptake into TFWT and OBT fractions followed first-order kinetics in foliage and green fruit formed during atmospheric HTO exposure. Such a pattern was not followed in old foliage, which had undergone some growth during exposure. The uptake data were fitted by a computerized non-linear least squares method to the equation $C_t = C_\infty (1-e^{-kt})$, where $C_t$ = tritium concentration at time t in pCi/g hydrogen, $C_\infty$ = steady-state concentration in pCi/g hydrogen and k = uptake rate constant in $h^{-1}$. Initial uptake rates, $C_\infty k$, and the time to reach 50% of the steady-state concentration, $t_{1/2} = 0.693/k$, were calculated from the computer estimates, all of which are shown in Table I.

2. The uptake of HTO into TFWT fractions of new and old foliage appeared to be more rapid than green fruit (Figure 2); however, with a high degree of variability shown as 95% confidence intervals, the rate constants overlapped. These uptake rate constants were 0.024 ± 0.023 $h^{-1}$ for new foliage, 0.104 ± 0.067 $h^{-1}$ for old foliage and 0.042 ± 0.136 for new green fruit. Larger foliage rate constants have been reported for tomato similarly exposed, 0.700 ± 0.140 $h^{-1}$ (Ontario Hydro Research Division Report No 83-33-K, 1983a) and calculated for bean, 0.58 ± 0.38 $h^{-1}$ (x ± 95% confidence limits, Garland and Cox, 1982).

3. The uptake of HTO into the corresponding OBT fractions of new foliage and green fruit (Figure 3) proceeded less rapidly than uptake into TFWT fractions (Figure 2) as expected. The uptake rate constants were 0.019 ± 0.018 $h^{-1}$ for new foliage and 0.010 ± 0.004 $h^{-1}$ for green fruit. A rate constant could not be estimated for old foliage because the tritium concentration in total organic constituents continued to rise, reaching a maximum value of about 28 140 ± 2390 pCi/g hydrogen between 1176 and 1512 h of exposure (Figure 3).

4. The steady-state concentrations for TFWT and OBT were used as the basis for calculating the ratios of tritium-specific activities with respect to the chronic atmospheric exposure source maintained at 70% relative humidity (Table III). The TFWT to average atmospheric HTO specific activity ratio was similar in new, 0.43, and old, 0.46, foliage. About half this ratio, 0.19, was calculated for green fruit. The foliage/atmosphere ratios were within the range of literature values cited in Section 3.1 (0.17 to 0.49), except for predictions of 0.77 at 70% relative humidity based on recent models of Garland and Cox (1982) and Belot *et al* (1979). Differences between model predictions and the present observation may be a result of the plant species studied, higher transpiration rates of clean soil water and/or leaf water compartmentalization. It is expected that ratios would vary daily under field conditions. With high transpiration rates during the day-time specific activity ratios would be lower than during the night when transpiration approaches zero in most species. It may be important not to generalize from either the present observations or the model predictions but to indicate predicted foliar TFWT/atmospheric HTO ratios vary between 0.19 and 0.91 depending in part on relative humidity.

5. The ratio of tritium-specific activities of OBT to TFWT in new foliage was less than unity (0.70) yet exceeded unity in green fruit (0.72 to 1.92). This is the first report of

greater than unity specific activity ratios obtained during continuous controlled environment exposures to HTO. These observations are explained on the basis of lower TFWT activity in green fruit than foliage rather than tritium enrichment in the fruit OBT fraction, since the OBT specific activities were similar in both new foliage and fruit. The lower fruit TFWT activity likely was the result of fruit receiving a larger proportion of comparatively clean soil water than foliage. These observations serve to explain the elevated tomato and cucumber OBT/TFWT ratios of specific activities reported earlier for produce grown in a prototype waste-heat utilization greenhouse located at a nuclear generating station.

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APPENDIX I
TRITIATED WATER UPTAKE KINETICS
IN TFWT AND OBT FRACTIONS OF TOMATO FOLIAGE AND FRUIT

RAW DATA IS SHOWN FOR TRITIUM CONCENTRATIONS
IN CHAMBER ATMOSPHERE, FOLIAGE AND FRUIT

| | | FOLIAGE | | | | GREEN FRUIT | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | UPPER | | LOWER | | TFWT | | | OBT | | | SOIL |
| TIME (HOUR) | ATMOSPHERE CONCENT. (PCI/ML) | TFWT (PCI/ML) | OBT (PCI/GH) | TFWT (PCI/ML) | OBT (PCI/GH) | REP1 (PCI/ML) | REP2 (PCI/ML) | REP3 (PCI/ML) | REP1 (PCI/GH) | REP2 (PCI/GH) | REP3 (PCI/GH) | WATER (PCI/ML) |
| 0.00 | 11394 | 79 | 0 | 53 | 0 | 3 | 7 | 8 | 0 | 0 | 0 | 2 |
| 0.17 | 11418 | . | . | . | 0 | . | . | . | . | . | . | . |
| 0.33 | 11009 | . | . | 161 | . | . | . | . | . | . | . | . |
| 0.50 | 10647 | . | . | . | . | . | . | . | . | . | . | . |
| 0.67 | 10581 | . | . | 502 | 282 | . | . | . | . | . | . | . |
| 1.00 | 16202 | . | . | 850 | . | . | . | . | . | . | . | . |
| 1.50 | 10091 | . | . | . | . | . | . | . | . | . | . | . |
| 2.00 | 9986 | . | . | 1466 | 1040 | . | . | . | . | . | . | . |
| 2.50 | 10105 | . | . | . | . | . | . | . | . | . | . | . |
| 3.00 | 10291 | . | . | . | . | . | . | . | . | . | . | . |
| 4.00 | 10329 | . | . | 2379 | 2213 | . | . | . | . | . | . | . |
| 5.00 | 10167 | . | . | . | . | . | . | . | . | . | . | . |
| 6.00 | 10037 | . | . | . | . | . | . | . | . | . | . | . |
| 7.00 | 10108 | . | . | . | . | . | . | . | . | . | . | . |
| 8.00 | 10090 | . | . | . | . | . | . | . | . | . | . | . |
| 11.00 | 10727 | . | . | 3366 | 3579 | . | . | . | . | . | . | . |
| 21.00 | 10708 | . | . | . | . | . | . | . | . | . | . | . |
| 24.00 | 11030 | . | . | 3049 | 3855 | . | . | . | . | . | . | . |
| 27.00 | 11218 | . | . | . | . | . | . | . | . | . | . | . |
| 45.00 | 11143 | . | . | . | . | . | . | . | . | . | . | . |
| 48.00 | 10841 | . | . | 4805 | 2988 | . | . | . | . | . | . | . |
| 51.00 | 10705 | . | . | . | . | . | . | . | . | . | . | 256 |
| 69.00 | 10255 | . | . | . | . | . | . | . | . | . | . | . |
| 72.00 | 10251 | 3730 | 18702 | 4482 | 3560 | 1714 | 2701 | 1498 | 16027 | 16818 | 17300 | 469 |
| 75.00 | 8461 | . | . | . | . | . | . | . | . | . | . | . |
| 93.00 | 13949 | . | . | . | . | . | . | . | . | . | . | . |
| 96.00 | 13983 | . | . | 5697 | 3818 | . | . | . | . | . | . | . |
| 99.00 | 13971 | . | . | . | . | . | . | . | . | . | . | . |
| 165.00 | 12157 | . | . | . | . | . | . | . | . | . | . | . |
| 168.00 | 11925 | 4913 | 35172 | 4754 | 4298 | 1577 | 2651 | 1574 | 20230 | 21451 | 13248 | 510 |
| 171.00 | 12160 | . | . | . | . | . | . | . | . | . | . | . |
| 336.00 | 9250 | 3920 | 33122 | 3800 | 6217 | 2162 | 1828 | 1414 | 22402 | 20401 | 29369 | 335 |
| 504.00 | 9335 | 4205 | 26172 | 4520 | 5811 | 1115 | 1574 | 1495 | 24001 | 26077 | 22169 | . |
| 672.00 | 9312 | 3996 | 27905 | 3827 | 6143 | 2084 | 1364 | 1806 | 21177 | 23925 | 21192 | 292 |
| 840.00 | 9862 | 4690 | 27389 | 4964 | 8302 | 2784 | 1835 | 1458 | 24993 | 28688 | 22047 | 205 |
| 1032.00 | 12956 | 5744 | 31839 | 5885 | 13911 | 4713 | 3032 | 2752 | 26734 | 25146 | 28169 | 301 |
| 1176.00 | 11518 | 4679 | 33155 | 5485 | 28486 | 3402 | 2305 | 2397 | 30658 | 25314 | 30108 | 207 |
| 1344.00 | 11734 | 5417 | 21572 | 6311 | 25590 | 4145 | 5730 | 2152 | 22490 | 28948 | 28719 | 429 |
| 1512.00 | 11096 | 4299 | 27222 | 6056 | 30332 | 3474 | 5136 | 4119 | 21680 | 31039 | 29299 | 284 |

CONCENTRATION UNITS FOR CHAMBER ATMOSPHERE AND TWFT ARE EXPRESSED IN
PCI/ML OF WATER AND OBT UNITS ARE IN PCI/GRAM HYDROGEN(GH) DRY WEIGHT.
TO CONVERT PCI/ML OF WATER TO PCI/GH, MULTIPLY BY 8.934



84-69

163153 RD